

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
28 October 2004 (28.10.2004)

PCT

(10) International Publication Number
WO 2004/091652 A1

(51) International Patent Classification⁷: **A61K 38/21**,
A61P 31/20

(21) International Application Number:
PCT/EP2004/003685

(22) International Filing Date: 6 April 2004 (06.04.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI2003A000826 18 April 2003 (18.04.2003) IT

(71) Applicant (for all designated States except US): **UNI-
HART CORPORATION** [IE/IE]; 41 Central Chambers,
Dublin 2 (IE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **TARRO, Giulio**
[IT/IT]; Via Posillipo, 286, I-80123 Napoli (IT).

(74) Agents: **BANFI, Paolo** et al.; Bianchetti Bracco Minoja
S.r.l., Via Plino, 63, I-20129 Milano (IT).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING INTERFERON FOR THE TREATMENT OF HPV INFECTIONS

(57) Abstract: Disclosed is a liquid pharmaceutical composition containing interferon for peroral administration, and the use thereof in the treatment of infections caused by human papilloma virus (HPV).

WO 2004/091652 A1

PHARMACEUTICAL COMPOSITION CONTAINING INTERFERON
FOR THE TREATMENT OF HPV INFECTIONS

The present invention provides a liquid pharmaceutical composition for peroral administration containing interferon, useful for the treatment of infections caused by human papilloma virus (HPV).

BACKGROUND OF THE INVENTION

Some 120 different types of papilloma viruses have been so far identified, which infect humans provoking an epithelial or fibroepithelial proliferation of the skin or mucosae and consequently warts and condyloma lesions. Genital infections, which in some cases give rise to neoplasias such as squamous carcinoma and uterus cervix adenocarcinoma, are among the most diffused HPV-related diseases. In a multicentric study carried out in different countries, the HPV types 16 and 18 were found to be responsible for 70% of the uterus squamous carcinomas, while different HPV types were associated with uterus epithelial tumors.

Papilloma virus infections are persistent and hard to eradicate therefore requiring a repeated therapeutic treatment and in-time monitoring of patients for relapses or development of pre-cancerous lesions.

Since the virus accumulates in the lesion sites, the choice therapeutic treatment should be aimed at controlling the diffusion of infection by removing warts or visible pre-cancerous lesions, by topical therapy, laser therapy, criotherapy or surgery. Such treatments, however, do not ensure complete elimination of the virus, which thus can start a new infective process.

DESCRIPTION OF THE INVENTION

It has been found that HPV-infected individuals can be treated with a liquid pharmaceutical composition containing low doses of interferon, to be administered by peroral route. The treatment according to the invention proved particularly

CONFIRMATION COPY

effective, allowing complete elimination of the virus with only few applications.

The invention therefore provides the use of interferon for the preparation of a liquid pharmaceutical composition suitable for peroral administration, for use in the treatment of HPV infections. As used herein, peroral administration means contacting the interferon composition with the oral or pharyngeal mucosa for a time sufficient to allow adsorption/absorption of the active substance and the stimulation of immunocells and cytokine secretion at local and peripheral level through the lymphatic system and blood stream. Suitable pharmaceutical forms include solutions, suspensions, dispersions, syrups or other liquid preparations containing pharmaceutically acceptable excipients. Water solutions containing buffering agents, salts and optionally stabilizers, adsorption/absorption enhancers, sweeteners, flavourings and cosolvents, are preferred.

The amount of interferon in the composition can range from 100 to 500 International Units (IU)/ml, preferably 150 IU/ml. According to a preferred posology scheme, from 0.5 to 10 ml, preferably 1 ml doses were administered one or two times a day, allowing a daily intake of from 150 to 15000 IU interferon. The daily amount can be modified depending on the severity of the disease, the general conditions of the patient, and other variable parameters. A synthetic interferon (e.g. recombinant) can be used, but the natural molecule, which contains different isoforms (α , β , γ) and subtypes, is preferred. Human natural interferon, preferably the α form, can be produced and purified from peripheral blood leukocytes or lymphoblastoid cells, according to known procedures, as described in US4732683; Cantell K. And Hirvonen S., Texas Reports on Biology and Medicine, vol. 35, p. 138, 1977; Zoon K.C. et al., Science 207, p. 527, 1980.

The peroral administration allows an immediate availability of interferon as well as the complete assumption of the desired amount thereof; in addition, it increases the patient's compliance and, of particular importance

industrially, reduces the costs for the preparation, storage and distribution of the product, compared to currently used interferon formulations.

The treatment according to the invention was tested in a clinical study involving women positively diagnosed for HPV infection, to whom a solution of human interferon- α (150 IU per dosage unit) was administered for a period of 90 days or more. The treatment resulted effective in gradually reducing the initial quantity of virus up to its complete elimination. Any difference in patient response could be due to the initial amounts of virus, to its genotype or to the specific immune response of the patient.

Details of the study are illustrated below.

EXAMPLE - *clinical study*

Ten female patients tested positive for HPV and subsequently confirmed as HPV-infected without immunologic diseases, were treated with low dosages of human natural interferon- α administered through the peroral route. A water solution was prepared dissolving 150 IU/ml human natural interferon- α in saline. The solution was stabilized with albumin and divided in 1 ml vials. For the treatment, one vial a day was administered for a period of 90 or, where necessary, 180 days.

At days 0, 30, 60, 90, 180 and 360 of treatment, a tissue-sample of uterus cervix was taken from each patient using an HC Cervical Sampler, and analysed with Hybrid Capture II kit and with 2HPV and CT/GC DNA tests (Diogene Corporation, USA).

Shortly, the Hybrid Capture II (HCII) test [Venturoli et al., J. Clin. Virol. 2002] is a liquid-phase hybridization assay utilizing RNA probes that discriminate 5 low-risk HPVs (6, 11, 42, 43 and 44). The DNA/RNA hybrid is immobilized on a plate by means of antibodies against double stranded DNA and detected by chemiluminescent-signal amplification.

The HCII kit was used for the detection of HPV in the lesion sites,

whereas dosing and semi-quantitative determination of viral DNA copies in the sample (referred to 100000 cells) were performed with a PCR-ELISA test (J. Clin. Pathol.: 1998; 143-148, as modified in J. Clin. Pathol.: 2001; 54:377-380).

In short, PCR-ELISA was carried out with a consensus primer (MY11-MY09) able to amplify 30 low- and high-risk HPV genotypes. The amplification products were labeled with digoxigenin during the amplification reaction, separately hybridized to biotinylated probes specific for 7 low-risk HPVs (HPV 6, 11, 40, 42, 53, 54, 57) and 11 high-risk HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59), immobilized on streptavidin-derivatized microplates and detected with immunoenzymatic assay (ELISA). A portion of the amplification product was analyzed electrophoretically to detect the amplified HPV which had not been typed with the available probes. Beta-globin was used as PCR-ELISA amplification control.

The assay provides a semiquantitative determination of the viral DNA copies in the sample based on the initial number of cells contained in the cervix sample and using calibration curves for each viral genotype.

The results are reported in the following table:

Paz. n°	HPV-type	HPV Day 0 Index value	HPV Day 30 Index value	HPV Day 60 Index value	HPV Day 90 Index value	HPV Day 180 Index value	HPV Day 360 Index value
1	6	11.000	5.300	350	-----	-----	-----
2	53	70	60	-----	-----	-----	-----
3	11	250	200	50	-----	-----	-----
4	6	120.000	52.500	400	90	-----	-----
5	6	20.000	13.000	150	50	-----	-----
6	42	450	250	-----	-----	-----	-----
7	11	250.000	110.000	800	60	-----	-----
8	6	2.500	1.650	180	-----	-----	-----
9	11	25.000	12.000	250	-----	-----	-----
10	54	900	600	100	-----	-----	-----

The Table data show that in 7 out of 10 patients, namely patients 1, 2, 3, 6, 8, 9, 10, at day 90 (end of the treatment) the viral load was undetectable, while 3 patients out of 10, namely patients 4, 5, 7, showed a significantly reduced viral load. Patients of the second group were treated for additional 90 days. At day 180 and 360 of follow-up control, all patients resulted HPV-negative.

CLAIMS

1. The use of human interferon for the preparation of a liquid pharmaceutical composition for peroral administration, to be used in the treatment of HPV infections.
2. The use of from 100 to 500 IU/ml interferon according to claim 1.
3. The use of 150 IU/ml interferon according to claim 2.
4. The use of natural or recombinant human interferon according to claims 1-3.
5. The use of natural interferon- α according to claim 4.
6. The use according to claim 1, wherein said liquid pharmaceutical composition is a water solution.
7. The use of interferon according to claim 1, for the treatment of infections of the genital tract.
8. The use of interferon according to claim 7, for the treatment of warts or condylomatous lesions of the genital-tract mucosa.

ABSTRACT OF THE DISCLOSURE

Disclosed is a liquid pharmaceutical composition containing interferon for peroral administration, and the use thereof in the treatment of infections caused by human papilloma virus (HPV).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003685A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/21 A61P31/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIAMONTI A ET AL: "Peroral alpha-interferon therapy in HPV-lesions of the lower female genital tract: preliminary results." LA CLINICA TERAPEUTICA. 2000, vol. 151, no. 1 Suppl 1, 2000, pages 53-58, XP009033655 ISSN: 0009-9074 page 54, column 1, paragraph 3 - page 56, column 1, paragraph 7 ----- -/--	1-8

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

14 July 2004

Date of mailing of the international search report

29/07/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Markopoulos, E

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/003685

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PALOMBA M ET AL: "Oral use of interferon therapy in cervical human papillomavirus infection." LA CLINICA TERAPEUTICA. 2000, vol. 151, no. 1 Suppl 1, 2000, pages 59-61, XP009033656 ISSN: 0009-9074 page 59, column 1, paragraph 1 - page 60, column 1, paragraph 13 -----</p>	1-8
X	<p>DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; May 2002 (2002-05), BARNARD DALE L: "Interferon-alpha. Amarillo Biosciences." XP002288399 Database accession no. NLM12090541 abstract & CURRENT OPINION IN INVESTIGATIONAL DRUGS (LONDON, ENGLAND : 2000) MAY 2002, vol. 3, no. 5, May 2002 (2002-05), pages 693-697, ISSN: 1472-4472 -----</p>	1-8
Y	<p>WO 97/31649 A (BROZZO RENZO ; TARRO GIULIO (IT); IFI ISTITUTO FARMACOTERAPICO I (IT)) 4 September 1997 (1997-09-04) page 3, line 5 - page 4, line 30 -----</p>	1-8
Y	<p>WO 02/36072 A (BIOMEDICINES INC) 10 May 2002 (2002-05-10) page 15, line 32 - page 16, line 32 -----</p>	1-8

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/003685

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9731649	A	04-09-1997	IT RM960427 A1	15-12-1997
			IT RM960136 A1	28-08-1997
			AT 205397 T	15-09-2001
			AU 722987 B2	17-08-2000
			BR 9707772 A	04-01-2000
			DE 69706657 D1	18-10-2001
			DE 69706657 T2	20-06-2002
			DK 886527 T3	04-03-2002
			EA 1147 B1	30-10-2000
			EP 0886527 A1	30-12-1998
			ES 2160927 T3	16-11-2001
			WO 9731649 A1	04-09-1997
			JP 2000506839 T	06-06-2000
			PT 886527 T	28-12-2001
			AU 2229997 A	16-09-1997
WO 0236072	A	10-05-2002	AU 2587002 A	15-05-2002
			BR 0115392 A	15-06-2004
			CA 2427194 A1	10-05-2002
			US 2002197235 A1	26-12-2002
			WO 0236072 A2	10-05-2002